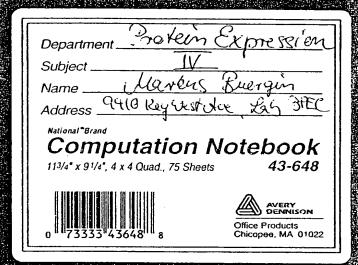
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Ruben EXHIBIT #55

alidorfordino portona calina Antigoria

Department To Hem (Subject IV Name (Markers Address 9410 Key With the	7
National *Brand Computation N 113/4* x 91/4*, 4 x 4 Quad., 75 Sheets	otebook 43-648
o 73333 43648 8	AVERY DENNISON Office Products Chicopee, MA 01022

Ruben EXHIBIT 2055 Ruben v. Wiley et al. Interference No. 105,077 RX 2055

1			1
	20		
-			
<u> </u>	•		:
•	12.1.95	I hangle from of CHO-dhfrcells	
	(2 //		:
i,-,		witz : 1) 3MP (1346 (BUP)	. !
	· :	1) 3MP (, M346 (BUP)	
			:
-4-	1 : : :	(M346 (TNE 28)	:
- j			
T·	<u>' </u>		
		11.28.95 CHO dute-colles confluent >5 flates	
		11.28:95 CM - Class Conflict 125 100 80	_
`-,		were shilled 1:10 and seeded in to	
-, !		the wells at a 6 well olish	
		2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	
·		00 000	···
	• :	(- resuspend cells of 175 in Oml	
<u>-</u>		dissucultur medium seed (me in earl crell at the Grele plake)	· · · · · · · · · · · · · · · · · · ·
_		in each chell at the Govern Acco	
<u>_</u>)
~-· ⁻		Coc.	·
T		mensare colle ocernight -> 90-959, conferen	ζ
	<u>!</u>		· · ·
=		- 11.29.95 - remove the medium of the 6 wells	
		= 4.005 may 14 M2	
		- yass colle with ASS	· ·
		- add I me transless. healing 1:	•
		MEM Lt, O'X OLES, 14 PS	
- :	<u> </u>		
- :[_	prepare slipotectin-Mix in 96 well plate:	
1111	•	property of the control of the contr	
		A: 90 re hondect. Medium 1 (6 wells)	
		10 jel Lipolochen	
417-	:		
171		8: 50 pl transfect Modern	
	: i		
		5 mg Expressionuecar (1134618MP, 11346/T)	46-75
		CHO-IITNES9)	
1 -		2 colleg for each con	06.04
:		The state of the s	1
.†		1110	
		118 pg 118	
1			:
Ţ-		- mix & +B: and masself 45 gt R	i
<u> </u>		The second secon	 '
+			_! i.
		- add transfectioning dispuise to the alsin	Hohow
J	<u> ! · </u>	in orbide Hor 4-61 W at 37°C, 5%, CO2	1 200
.1-		31,00	
<u>†</u> – _	1 1 1	add (me transfect , had 2 to so s) in lead	
-	.~		
		Jou Gwell Dist , WEN LT, 10% OHBS, (7, DS)	

1	•			1 . :
		· · · · · · · · · · · · · · · · · · ·		37
<u> </u>	Stabely fouglocled (NG colle -	CHO/THE X	1.1.3.95
1	. 3. com normez		. :	
				1 1 1
-	Cells were panelected	94 12,1,99	5 (500 7.20)	
31:	and seeded into they	inidoma places	for selection	: : :
	(se p.21)	·		
£1 :		· · · · · · · · · · · · · · · · · · ·	· · · · · · · · · · · · · · · · · · ·	
\$!	Cosenations:			<u> </u>
1 <u>3</u> 1		1 10	0 0-	<u> </u>
<u> </u>	- for all 3 consh	CAZ Arené copre	Ma Clones Kolino	<u>.</u>
<u> </u>	Sen colle were	e socoled in me	dun Het Confuned	
	: Oome wix	.		
-: · ·	Jup/11346 . m 4	are Sub dama	note Done mulch	•
	- BUPINSYE on the	ree riginion in	being the order	• •• •
	20ml rix only		mund.	
· ·	- clave	reck you ger	leneal)	
	-XXXXXXX	Min Eduna	Comest	: : :
71				
स्र	:- TUE 8 (N346):			1 : : : :
		. <u>J</u>	;	
-\ :		. 2	<u> </u>	
		3	<u> </u>	
-	<u> </u>	;	6	
*	71.720 (01/0)		(0)	
	- TUPS (CHO)	<u> </u>		+ : : :
		<u> </u>	·	
		:		
-		7	LV .	1 1 1
		<u> </u>		1 1 1
	1 :			1 1 i
	the hyprolous cells	coits osbros a	pre labeled vis	
	a marker	<u> </u>		: ! ! !
. 	1		and the plake	\mathcal{L}
	- Legue caller modern	ces remoded	sing the warm	<u>- </u>
	- Lessusceller meden		and the prairie	
	- Lessus cultur medium		and the part	
			i i i i i i i i i i i i i i i i i i i	
			and the part	

		` `	
Temening PBS coas remoc	ed ant all		
- the wells that contained	Cano as	· · · · · · · · · · · · · · · · · · ·	
a zone Ripet	ا دیمانید	me mong	<u></u>
			<u> </u>
	:		
- 7 pl of Juppen was add	lea yo the	wells that	i
confound clones and	sells were	merbeled	
dor 5' at 32° e			
2000 00 0000 1000	- 	سالسانسروند سرک رد	
- alls of sad well were well of s	Bangkenec	luxo xe	
(ime MEM L, 5% aft	Se in De	Day to want	- 00
	3, 6, 63,	WAY FILXIO	sell _
:			: -
9.96 - Osservation at grow Raf	2000	90.000 0000	
	ب برد	- chest outil	25
WED single colle	2.1		
1.2 hangler colly with 6 well origh	2.2 2.2	: :	
HO 1.3 > 6 well out 5	2.3	> 6 cele dig	5 1
20mm Mix 1.4	2.4		
1.5 S.C.	2.5		
1.6 -> 6 well of § {	2.6		:
	2.7	> stansfer colles	6[25]
	2.8	<u>S.C</u>	<u> </u>
	2. <u>9</u>	3.C	
1.10 S-C.	<u></u>		:
31			
3 2	· · · · · · ·		
3.3 60			<u> </u>
3.4			<u> </u>
3.5			
3.6			<u></u>
3.7			· · ·
3.8	<u> </u>		· · · · · · · · · · · · · · · · · · ·
3.9 -			· ·
3.10	· · · · · · · · · · · · · · · · · · ·		
3.11.			<u>:</u>
		1	
		· · ·	

		: :	39
			
· · · · · · · · · · · · · · · · · · ·			
TUE 8	1.1	S.C	2.6
1,346	(.2	S.C	28 -
12 mMMX	Ent.	:	2-9 -
	2.۱		2.00 -
	5.3	S.C.	3-2 -
	2.4	S. C	3.3 -
	2.5		3.4
:	3.5		
·	3.6 3.6		

19.96
- CHO/DG44 calls: I was vias viaced and socied.
TEN 1 - Modern 5% OFBS.
MOUNT
1x.42 CHO/DG44 O1.09.96 - 41
VIGE 3: I wal was thanked and souched into 9 775 land
(100, 11) in MEM 2 - Modum 5 Y. O.FBS, 17.PS, 50, MMIX
VIGF 3. 01.09.96-0
VIGES: Luce was Krewed and souded into a 125 tags
VIGES: (will was trained and seems.)
(5,41) en MEM 2 - Moslum, 5'/OFBS, 1'1.PS, 5,41 MTX
VIGES 01.99.96-B
CHOIDG 44 cells. Dassage celle = 1:4. m/o 1.12.96
CHOTOG 44 colls prosing some 7 >5 forst or CHOTOGUY Orange 5
one (>) atomic
1 1 1 1 7 7 10 060
11/1GE3: passage celle 1:4 into 4 7:75 plasée
(100 pm) 1112 04.00, 96-1
VIGES passage solle list into one 775 last
1 (5/4) Passage coach (1) 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1

:	The facility Pic	
	1-4-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1-1	<u>#</u>
•		
: : :		
:	Markus Work (Markus - Sick)
	11.5196.	
9	or in the second se	
	b: oopM. cHolaff	eno/dhfr
Š	0644 (1-73) > D644 (T-245)
		1/A/76 - 6
A Tribut	· · · · · · · · · · · · · · · all	Pf coltine-pellet-
	· (<i>47</i> .5	me NEH-0+ + 12 ps (11/5/9-1)
	ት አ.ሮ	me HiFBS ((10+. 16 000-044)
		(10+ asp.4546-)
9 2		GIBLO
	VIGFS T-7	TX) -> VIGES (T-225)
4	1.9.96 (Syma	1/15/96 (SUMMTX)
		1
3	· · ·	of culture pellet
	ە كىرى	me Mom- = + + 5 D; FBS +18ps
	1 0	ith SUMMTX (1/5/96,82)
	1	
<u> </u>	· · · · · · · · · · · · · · · · · · ·	I'ME of south atx stock)
		7-23- TAF-Y-CHO-T-72-
		20 nm) -> 1/1x/96 (20 mm)
	(9	11 of culture rellet
	, , ,) ,	rom/ NEM-2+520;+B1+12P1
	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	
	. The transfer of the transfe	Wit 20 MMTX (1/5Al. H)
1 1 3	<u> </u>	-8 ml of COOMMIX)
	10 m	HOME & TS & DI FBS +1 &PS
		L COMMMIX
	10 mg	1 JOHNHTX 1/6PC
•		
	16 T 75 Dasho 1/10 F2 6	10996-1 Subini Hea lo
		include 1 Sitalum where the
<u> </u>	June Fring	

	41 .
	<u> </u>
- SI 5, SI 10 cells: Omparatione Study	11.16.96
- de como e exmession ofter source Descres	
- la compare expressionland after swere passages STS and ST 10 alles were trawed and seeded	
MEM L 5" a FBS, 1"- PS, 80 MM MTX	: :
	
S15 - 01096 - 90	<u> </u>
ST 10 - 01.1696 - PO	
5.20	
TNF & clones (colls translooted with CHOOI U	60 (dv.)
con a Contract and in Tage	•
cells from 6 well dish, passage into 725	•,
TNF >, CHO, 1.2	•
TUE >2, C40, 13	
i	
colls from 24 well dist, passage into 6 well dist	<u>.</u>
71:- 79 (3/0) 1.00	
TNE-2 CHO, 1.10	
TNF8, CHO, 2.3	
colles from 6 evell dist passage into 6 evel dist:	
TUF 7. CHO, 1.6	
THE CHO 19	•
(01.2)	:
	:
	1

₹* ! !		
1.17.96	TNF3 - CHO cell lines theirskeafed at 11.29.9	7
	and transfered into solutive medium at 12	,
i	- Several more dones were pieded from hybridona per A	60
ži 	into 24 ciell olisses:	5)
Ē		
<u> </u>		
4: 	INF8 (cells were translacted with the Cho1 weeker	
į ()	INF84.1/ 11298/ POI to TNF84.4/112995/POD	
2)		
3	TUEY 6.1/112295/ POI 40 TWEY 5.10/112995/ POI	
1	1	
	The 82 (cells were transfected with the 1346 world)	
: :		
7)	TNF > 4.1 / 11299 = 1701 & TNF > 4.10 / 112995 [PO]	·
5)	THE 7 5.1 / 1129 95 1981 \$ THE 2 5.4 / 1129951 POLL	
F=		
		
	- medium was removed from the hybridaya plates	
	- Sylvidana plato cos cossed with 10 mil PBS	
	- BS CDS Newwood	
-	- the wells that were marked and numbered les handlet	
	lidere, were alequest for 7BS using a 20 pl Pipperra	
	and a india expass shall yellow the	<u> </u>
	-5 wells were try primited at one time	
	(to onhall line of made vila trupan)	
	5 incubición at 22°C	
) MISS ACTO 3 - 20 - 3/2 C	
	- 10 pl of medium cas added to the coell	: 1
	alls were tamofered and sorded into the	· · · · · ·
	coele of a Discoele plate in	
	WENG SIGHTS (N. HS, SOMM MIX	:
	(usung si 'Land 'Ripettermour	: :
# 16		. ,
\$ K		-:

122.96 The y-clones - Calls expanded in T25 fastes
(CHO: well were pareticled with Caboi derived vector) +20 mlk MIX)
1.) (40/TNEY 1.3/112995-864: 1725 conferent on 2725 \$66529)
- 1 1000 FTUE & 2 7/11/2995 -984 : 175 conflue-i 1:10 m 1/15 plage
3.) CROTINE 7 2.11
0.725 10060
4) CHO(TNF1.6 (112995-PO4: 1-6W confluent in 2 7 25 flagges
5.) CHOLINE & 1.7 (112995-704: 1.6in confluent in 2 725 flages
6) CHOLING \$ 1.9 / 113995-904: 1-64 confluent in 27 25 pases
27.25 10.25
7) CHO (Thir 3 2.3 / 112995-PO3: 1-6W conflict in 2725 \$0.565 8) CHO (Thir 3 2.9 / 112995-PO3: 1-6W 40% confl. in 2725 \$0.565
8) CEO (14) 35.4/ (1244) -403; 1-800 481.0ml. 14 51 51 2-413
West steps with this clones:
- all clones split in 2 T 25 dests, one last of each alone
collègents et 1-106 collègent Collègent the second T25 collègents et 1-106 collègent Collègent the second T25 flags are gaing table secoliq in 6 wells of 4 6 well of the at
latiques at 1210 constitution of 6 well of the at
0 20, 50, 100, 200, 500 ml MTX for amplifies for
<u> </u>
1.23.95 Comparative Study ******** 346 ST5 and CHOST 10.1
in hEM L. 5% afro 1% PS 88 M MTX
- Thiday 1.19.96
- STS 011696-700 > POI : HAR content T75 past split 1:3 into 3 1 75 flasts
515 B11646-700 >101 : 1×907. confered 725 fait spect 1:3, into 35 25 AQ 269
- 3 × I'me aliquet at bord clones were hosen at -80°C:
- Tuegolay 1.23.26
1 75 Marko are on 90% confluent 1 77 least of each
cell line was given to Alarie Collins for long terry comparison
in grimes faixes
N
- 3. I'ml aliquets of both cellbris were from at -80°C

<u>.</u>...

	- cells at 1 ST 5 and 1 ST 10.1 729 this were
	passaged into 2725 test at a spatials of i.3 792
Eg(ablishing of stable CHO-TNF & clones 1.25.96
(see	clones were translacked with the CHO-Oloperioed Cooler)
	clones: 1) Chalther 1.3/112995-Pay=105 2 contains 125 facing
	2) CHOITHER 1.2/112995-PO4->PO5 2 conferred 725 flasks 3) CHOITHER 2.7/112995-PO4->PO5 2 conferred 725 flasks
	4) CAOITNEX 1.7/112995-PO4->POT 2 conferent 25 floor
	5) CHO(THEX 1.9/11.2995-904-3PX 2 CONTENENT 725 PORES
	All the clones:
	first 725 flast: - \$250 a 2 ml aliquet of the TCM
	for toding in a western assay.
	trooving. - trypsenise colls and resuspend in 16 ml final colume seed 10 ml in (1:1.6)
	(75 flass (NEW) 5'2 degs (XR TEMMACK)
	- freeze prese colle on Juegolay
	- colls of T75 TOF 8 2.7, speit 1:5 m T75
	Second (25 flags of every along)
	- Appenie calls, and resugned in 2 ml MEAL, 52dfls, (Y. PS
	- Soud (ml in a T25 last in MEAL 5" OFB) (Y.PS 20-MA) mext day replace medium with CHO-SFM-II + 12 PS:
Jest	in autobe occar WE and Sanoust somples for Western +
	GO resuspond semening I'me in a tinde cooleur of 6 dul
in ding	Mer K, 5, a fB3, (N, BS, O MCK)

	: :
A STATE OF LOCAL CONTRACTOR OF THE PROPERTY OF	
7.) 346-TWFY 9.7 /112995-981-02	
8-) 346-TUFY 4.10 / 112995-POI-02	
9) 346 THEY 5.3 (112995-POI-02	
W.) 346 TNFY 54 (112995-POI-02	
•	
1.27.96 Establishing of slasle CO-Thit's - clones:	
7	
Clones for Jesting in Western blots and in it Da	4
Boolmants Casacs:	
The state of the s	
1) (HO THEY 1.2 1112995 - POS	
2) CHUTNEY 13/112995-PO5	
3) 17t0 TUE & 1.7/ 112995-PUS	<u> </u>
9) CHOTUFY 1.9/ 1129:95-POS	
2) CHOINER 5.71 (15662-502	
cells were smaked into 125 flasses on 1.25.9	(6 (p. 45)
	
- Exdange Dre dissue culture midum	
- remove medeum	· · · · · · · · · · · · · · · · · · ·
- 4959 Levice with PBS to mose gave to your	26.6
all tacks of MTX - add 5ml CHO-S-SFM il + 17. PS and	
	<u>^</u>
incubate for 2 more days	
- ds nightive combal for the 2589 48 also change	10
	re
modern to CU-S-SFLEX + 1:2 PS and for clone of	
ST10.1 1011696-803	
1.2. 96 - hancest supervalents of clames a socie and aliquot	
east saple in 5 x Iml aliquate	
- freeze down 3 aliquois Colone for westering	
- Suntained Months You Continued.	.:
Submitt 2 x 1 ml aliquot / clone do Dar Bedman	lati :
artivial asser.	
The state of the s	

:

STATEMENT A

ŧ

	•
	49
	:
Establishing of Stable CHO-THEY clanes	i.30.96
725 flagby Condenset: => speit 50" => 775 hearing, 25" > 725 drawing & 25 x -> 6 well amplifies	• :
25% > 6 well amplifies	ka
(ill claves harstacled with hanglacted sits les p CHO 1	· i
(pC1) derived vector	•
7.) CHO TURY 1.6/112995- P. 04- POS	
2.) CHOTERY 2.3/112925-7 04 - POS	<u> </u>
3.) (40 1 NEX 5.4 (115442-604-602-	
4.) CHOTHEY 6.4 (11 29 95 - POZ - POZ	
2.) CHOLMENET (115602-603-603	
6.) CHOINFY6.8/11295-POZ-POZ-POZ all collling an	
confluent in T25.	fuit
- remove Modern han last	
wast cells will PBS	
typswise soul hyper 5 37°C	. 90 0
- resuppose in 2 ml livel value MEM 1, 5% aFBS, (NAS ONIX
<u> </u>	
1.) Seed I'me of the cellsusp. in a 175flag in 20.	ml
Joelring MEMI, 5% OLERS, 1% PC, 20 MM TX So expand	· · · · · · · · · · · · · · · · · · ·
coles for freezing of selected elones	
2.) Socola5 ml in Stul MEML, 51, 0 FBS, 14. PS, 0)	M(X
into the first well af a 6 well plate, mic well and	:
good I me at the cell suspendion into each well a	<u> </u>
Utemplifie dre 6 ciel plate	,
- add 0/50 pe / 250 pe / 250 pe / 1500 pe	
of MEML, 5% aFBS, 14.PS 800 MM MIX.	<u> </u>
- adjust valeur de 2 ml each vell	
=) litte endersonhating 0, 20 mm, 60 mm, 100 mm	
700mm 400 mm.	
	
Johns B.) Seed 0.5 ml at all apparain in 6 1725 plans in Johns MENL, 20 ml MEX, 5% OFRS, 100 PS	
- Gange Head when calcare confee of the CHO'S-SENT WEE	2-1-1-2
	1 1 1 1

50			
	- incubate for 2 more days, then Sances to medium for texting in Gestern + whiteviral classes		
	Eslay lating of stable CNO THE & clones (pC1-constr	10 fg)
130.96	heering of improcess cells after clonelselection in hyphistomaplates, expanding at alones in & 24 well, toward, 725 and 175 flates in presence of 20n M Litex.		
	(continued from P. 95)		
	2) CHOTHEY 1.3/112995-POS-OC 4) CHOTHEY 1.3/112995-POS-OC 4) CHOTHEY 1.3/112995-POS-OC 20 MM MTK	<u>:</u>	
1 . 1	- typsinge cells and resuspend in Conl		
:	MENT- 5% OFBS- 182-DM-50	 	
	heere dan 3 × (me aliquots af each abre here in Malque heerig box at -80°C over night houlds alls into the -(40°C preser		
130 QC	CHOTHEY- clones: expanding color for freezing	-	
	Celesare conflict in well of a 6 well dist. pessage colls into a T75 lastin MEN L 5 COEBS (CPS DOWN MTX	!	
	3) CHO LMED C. C. LII SAAZ - 605 - 03 5) CHO LMED P. 10 (115542 - 603 - 01) 1) CHO LMED R. 100 (115442 - 603 - 01)		
	3) CHOTHE & CO/42295-802-63	· ·	

		•
		51
	: Comparative Strate 346 STS and CHO ST W. 1	130.96
	take 3 = Int aliquois of oral elone and passage	
24.35	the coles in MENL, 51. aces, 1278, 80, 4 Mix	
	Speit to lio 1:3	
	1) 346 575 / 011696 - 703 - 04	
	CHOOLE 1 Day 4 cells	(30.96
	Pro-	<u></u>
·	Passage	;
- : :	G100149- 1844/010996-709-10 Speit 1:10 secol	s tare
	LIEML+, 5% OFB3, 1% PS	
	Establishing of steple CNO-Third clones.	13196
	Havest of medium for lostinger westeng and in	
*	anti beat assays.	
	1.) CHOTHEY 16/112895- PG5	
	2.) CHO THEY 2.3/112995-POS	
	4.) CHO TOF 86.4/ 113995 PO3	
	2) CHO LIPE S. C. S. 11 58 52 - 60.5	
	T25 plasts 90% conflict	
	12) Justes 40%. Complete	
	- nenew /c-medium	
	- 425 2 x sits PBS	
-1-1-1	- incussion los & more others	
	- in Cr. Sa Co. Lot & more about 9	

*	1.
	53
L, , . ,	- seld cells in 6 wells at a Gio, aist, Indicate
i izvišeni i	- 200 0, 100 pl 200 pl 500 pl 800 pl 1000 pl
	1 of cooper rix to general o 10 H 20 m M 59 m
- 1	80, 12 2 00 h M MIX (MENL- 5: 068 12. 18, 200, MARK)
	- adject wells to 2 me final cooline (well
	(MEM), 5% ates, 1x PS, ONTX)
	Establishing of stable CHO-THEY clones (perconst.) 2.1.96.
	- C3 1.2.1 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7 7
	howing of in provess colles after clonal selection and expanding
. 1	of aboves in T75 touts in presence of 20 mm mix
	(Continued from P. 49)
	1) CHOTHEY 1.6/112995-PO6->07
	2.) CHOTHEY 6.4 / 115642-603704
	3.) CHO TUFY 6.7 / 112995 - po3->04
2 1 1	4.) CHO TUFY 6.8 (112995- po3-204 (20~MMX)
6 -	trypginise cally and nesigo in and
*	- Som 10° 81 800 RPH
	- resugged in 3ml treezing maken (JUHL, 5% af BS, WY. DAS)
1	greeze down 3 x 1 me signores of each alone
	treeze in Holgere treezing box at 80°C acraiget
	- transfer celle en -140° C freezes.
	Eglablisting glaste 346-The V clones (ph346 constricte)
	Eghblisting globe 346-THE Y clones! (ph346 conistricte)
	expanding of clones for hearing (backers!)!
	1 Cell res grown on 24 cell of res (see p. 42)
	1.) 396 (DF & 9.2 / 1829 95 - POI - 02 - remove TCM sygenise
	5.7 26 105 21 d. 81 11 51 1 500 1 51 1 1 1 1 1 1 1 1 1 1 1
aparete in	5.) 346 TW 81 4.6/112995-1991-02 - newsp in 2ml elect 1 570885
	1) 346 LNEX 21/11/6/42 - BOLOS: 1 1/38 50 MY YOU
	5) 346 This of 5.2 (11,2995- Pai -02 - good in Egill
	<u> </u>

_		
2.1.46	Eglablising globe 346 TNF8 cloves (pU346 denie	ec()
- <u></u> -	Several more clones were picted from hygridones palo	S
	into ?4 well alighes:	
- :		
- <u></u>	人) 346 That 6.1 (112995- poi	
·	2) 346 TNB > 6.2 / 112995- pol	
	3.) 346 TNP86.3 (112995-p01	
	4.) 346 TURY 6.4 (112995-pol	
<u> </u>	5.) 346 [NAY (.5/11 2995 - PO]	·-·
	6.) 346 INFX6.6 (113995- POI	
	12) 346 TNF36.7/ U2995 - POI	
	18.) 346 TNF 868/112995 - POI	
	(9.) 346 THE 86.9/(12895 - PO)	
-	6) 346 TNF36.10/113995- pol	
·· ·		
	Steps see protecul p. 42	-
	1 Steps see promote p. 72.	
: :		
	Establishing stable CHO TNFY clones (PCI derived	,
	Establishing stable CHO TNFY clones (pC) derived	<u></u>
	1000000 de 600 0000 000 000 000 000 000 000 000 0	
	clones showere growing in 6 well distres or 24 well alistes	
-	were passaged into 6 well alistes in MEM 1- 5% OLFRS 20	MMIX
•	17. PS (Colls are going to Se exproded into 725 lass los	<u> </u>
	freezing of backup clares)	
	1.) CHOTHEY 5.1 (112995-POI-02.	
<u>:</u>	2) CHOTNEY 5.2 (112995-POL-02)	
		· · · · ·
<u>_ ' ' ' '</u>	3) CHOTUFY 5.3 / 112995-p01-02 / hom 29 well	:
	4.) CHO FUFY 5.4 / 11 2995-po1-02	
— <u> </u>	S.) COO TNESS-6 / 112997-POI-05	
		: :
<u> </u>	7.) CHO LNES, 6-1: 11.5662- bot-05.	<u> </u>
!	8)1 CHO THER 6.5/112995- POI-05	
	9.) CHO KNEY 4.3/112995- poz-03 }	
:	10) CHO FUE 2-5.7 /11/29 95-03 / 100 Cold	 -
_ ! ! ! !		
	2'(26	' :
.		
		· · · · · ·
	· · · · · · · · · · · · · · · · · · ·	

		55
	clones that were growing in 6 well divises were prossignation to	: 2. r. 4 &
	TIE TIS 1008 IL MEM J - STOCKS (PS 20~1)	7:×
7	[25 or T 25 lasts in MEMI, STOFFS, I'PS, 20 NM (expranceder T 25 lasts for homis ex booky claves	
	1005	
	1.) CHO THEY 4.1 / 112995 - POZ-03 -> TZS	: , ;
	5) CHO LNES 4.5 (115642 - 605-03 -> 152	
	3.) CHO THEY 4.4 (112995 - poz-03 -> 725	
	2) CHOTNEZ 2.9 (1158 St - 605-03 -> 122	
	// (140 / 131 / 2 / 1	<u> </u>
	Estatisting of state Coio clones TNF8 (pCI constr.)	5.5.66
## 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	I are a soli accompany of a flow a come a secretion and areas	d to
2	processed after processed after about setection and expende of 20mm MK	·
	(continued from p 45. 29 49)	
		: ! !
	J.) CHOTHEY 2.3 (12995- P.06	
	3) CHOTHEY 3.3 (112995-p05	1 :
Marie (Marie)	(4) CHOLVIE & 6.5 \ (15402-604	
	5.) CHOTHE & G. 6 / 4 29 95-po4:	
	6.) CHOTUE & 6.10/112995-POY	1::::
		1 : 1
	trypginge colleg and regisp. in come	
	Spen 10 get 800 npm, negusp. cells in 3 ml.	1, 1, 1
	booring meaning (MEMJ-, 5% of BS, (O'r. DAGO)	
	priese down in 3 x (ne alignat soot alone in Utalgeria	++++
	thousing look at -80°C good might!	
IN THE	pangles alls in - 140° moser	
		+
		++++
96	0.06	++++-
		1 1 1 1

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i

 	2296	Establishing of stable CHO THE & clones	:
 	·		<u> </u>
·		harvest of medium for lasting in coopen- and	
		artivital assags (continued from p. 51)	
		- after 2 days of incubation in CNO-S-SFM-II + 1% (<u>i</u> q -
 -		the medicin was beneated and horar in	<u></u>
		5×1 ml gliquets ouch abone at -20°C	
		1.) COTHER 1.6/112995 P5	
		2) CHOTHER 2.3 (11.2994 p5	!
	i	3) CHOTOEX 29/42995 , 5	
		7.) COOTNEY 6.4 (112995 p3 5.) COOTNEY 6.7 (112995 p3	
		6.) CAO LIVE S. 6.8/ 115662 b 3	
 	0.2.06		
	22.96	Camparatile Study CHOST 10.1/346575	
		date 3 x lml aliquots and clone and passage	
	1	fre colle in MERI, 5% OFRS, LY. PS, 80, MIK	
	<u> </u>		
: 		1.) 346 315 / BILGG6 - PB4-05 SPRY 1:4	
-		5) CHO 2001 (011858-605-02-28-1: 1	<u> </u>
<u>. </u>	: ! :		-
<u></u> -	!	79582949 of CHO dul- 1894 cells	
		4988949 OF CHO - 10394 cells	
		160	<u> </u>
<u>:</u> -		CHOCKET DR44/010996-p11 906+ 1:10,	 -
.—-~ :			
.— —-		good one T225 Lase in MFM1+5% alfBS, (1. PS	1
نــــــــــــــــــــــــــــــــــــ			
		2245	
			- ; ; .
-			
<u>-</u>	.! !		
			- •

Belga!	1	
		57
Eligania	Establishing of stable CNOTHE & clones	15.5.66
		12.5.76
F 产生。	Hearing of Clones (hackup), in processally	
	alla claral selection, and expendio in 775 lasts	
Potent I	9+20mle rox	1
34.57	1) (10) 71 = 9/ 10)	
	1.) CHOTHE 71.10/ 112995 - P. 05	
	2) CHOTHE \$ 5.5 / 112995 - 8045	<u> </u>
	7) C40 (6(7) 5.4 / ((243) - 704	: .
	l : .	2.5.06
	Comparative Stroly CHOST10.1-396575	2.7.00
	5-19-5-10-C-51-5-10-5-10-5-10-5-10-5-10-5-10	
	9 1 1 1	
	Forering of clones for further analysis	
1-1-1-1	1.) 346 STS / O116Q6- pos-06	: : :
	2.) CHO STO 1011696 - pos -06	
	Passagua of CNO Olate / DE 44	760
18.4	· · · · · · · · · · · · · · · · · · ·	2.6.96
	CHO dhe Da 44/010996-pm 12 spect 1:10	
18	seed one Ters last in Men 1 , 5% of ERS, 1% PS	
	1	
		2.6 96
	Comparative Strong CHOST W.1 - 346 STS 1	10.20
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		
	date 3 x (ml sliquois each clone and pessage the cells	
	dobe 3 × (ml sliquois each clone and passage the cells im MEM L - 5% OFBS, 1% PS, 80 mm MX	
	date 3×1ml aliquois each clone and passage the cells in MEM 1 - 5% OLFBS, 1% PS, 80 mm MX	
	m MEM 1 5% OFBS, 1% PS, 80 mm MTK	
	10/2 3 × 1 ml slignofs each clone and passage the cells m MEM 1 - 5 × OLFBS, 1 × PS, 80 mm MMX 1.) 346 STS / OTTG96 - p. 05-06 Split 1:3	
	M.) 346 STS /011696-p05-06 Speit 1:3	
	m MEM 1 5% OFBS, 1% PS, 80 mm MTK	
	M.) 346 STS /011696-p05-06 Speit 1:3	
	M.) 346 STS /011696-p05-06 Speit 1:3	
	M.) 346 STS /011696-p05-06 Speit 1:3	
	M.) 346 STS /011696-p05-06 Speit 1:3	

26,06	Establishing of stable CHO TUF8	
	Expanding of many and and a second of second	
	Expanding of cells for freezing, passage coles from 6 wells to	
		:
	2) CHO THEY 65/ (1299) - p02-03	
- i	3.) CHO THEY 5.7/ 112995 - p03-04	
	4.) CHO FUFT 5.4/ 112997 - p02-03	
	Expendence of color for former of the former	
	Expanding of colls for thereing, passages cells from T25. to T75 loses in 20 mm MX (hereing backupsaney)	• •
	() CIOTA E 2011 (1200CT 08 01)	
	1.) CHOTHE & 4.1/112995 - p03-04	
	3.) CHO TOE 74.4/112995 - p03-04	
•		·
	Establishing of stayle CNO TWP8 clones	
	Unplification: continued from page 52	
	Continued from page 52	
	calls confluent at 200 ml and les alleg at 400 mill	_
···		-
	5) CHOENEN 13 \ MS662-608-02	· <u> </u>
:	3) CHO THEY 1.7 / 112995- POG-02	
	4) CHOTHES LA / (12995 - PR-03-	
	2) CHOLME > 5.5-1 (15602- bac-05-	 :-
	- remove JCM 51287 47 B (ml PBS/Gell.	<u>:</u>
	hypsinge 5' 200 pl Japan (DAPA (well	-
· · · · · · · · · · · · · · · · · · ·	(only 200 m M. and Good Missell	
	- poole colles of 2 wells and rosuspond in 6 ml FV m	
	MEM 2 - 54 OLFBS IN BS ONTX	-
		<u> </u>
· <u> </u>	is seed the cells unto 6 wells of a new 6 well dist mel/well	I
·		

		59
	8. 800 (
	- add 0, 100, 200, 500, and 1000 pl of 200 pl	
	MENL 51. 1889, 17. PS, 2 print de de veles in ordes	
	10 golice 0, 100 nm, 200 nm, 500 nm, 900 1 nm	
	MX final concar hather	
1		
Wilder.	add WEHZ (5) alos (1) PS, OMX, adjust finel	
[]	colume to 2 ml (gell.	·
	Esteblishing of stack CHO THE Yelones	2.6.96
	Course of sure	2.0. 6
12 444 -		
	Muphiliaghan: continued for p. 49	
		:
	colls govin (wells 20 - 400 MM MTX	
	1.) CHOTUEY 1.6/112995- PO5-06 pools 100,200, COONIN	• - ·
is in the second	02 00 20 2 0 2 / 112460 200	···———— · -
	2) 000 N.D. 20 (112905 DIS-05	
	(1) CHO LNESS (16 (((5) 18) - 62)-04 Doops 500' (000-74)	1
	5.) CHO TNF8 6.7 (U2995 - FO3-04 poole 100, 200, 400 mm	
	() CNOTHER (8/ 113992 - 362304 pool 200,400-1	:
	remaie TCM wash with I'me PBS / well	
	trypsinge 5, 200 pl Trypsin EDTA (well (wells the same	
	poole cells and resusp. in 6 ml final colume in METY 5% at BS	5/262
2 1 2 4 2 1	OMTK	
	Sand colleg in 6 wells of a Givel dist und (well.	
		2.00
自己有了	add 0:50, 150, 250, 500, 1000, lof MBUL 5% dEBS,	
	Concertation . 20, 60, 100, 200, 400m MTX for	
	add MEMI, 5% OLEBS 1% PS, OMIX adjust divel	poline!
	yo 2 me / ciele	
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1		
	 	
		

:

Freezer Box1-1

	In Process	Cells	·				
							
_	Recombinant Co	ell Line:	CHO/pC1	/TNF+IL6			
	Date:		02.07.96				i
	Clone ID:		HLTBT71		i		· · · · · · · · · · · · · · · · · · ·
	Project Code:		HG10700)			
	Rack Number:		1				
	Shelf Number:		1			-	
	Box Number:		1,1				
	Freezer:		Equipmer	it Room/BPEC			
	Host Cell Line:		CHO dhfr	- (ATCC)			
	Passage Number		-	,			i
·	Expression vec	tor:	pC1/TNF	7-1L6			1.7
	Construct made		Guo-Lian		•		
	Date of Transfe		11.29.95				
	Cell Line Establ	ished by:	Markus B	uergin			1
	Notebook:		443				i
			<u> </u>				
	Clone Number	Passage Number	[MTX]	Freezing Date	Location	Tested	Amplification
		· · · · · · ·			(in box)		VpGado.r
1	CHO TNFy 1.2	112995-P06	20nM	01.30.96	A1-3	AV	+
2	CHO TNFy 1.3	112995-P06	20nM	01.30.96	B1-3	AV	+
3	CHO TNFy 1.7	112995-P06	20nM	01.30.96	C1-3	AV	+
4	CHO TNFy 1.9	112995-P06	20nM	01.30.96	D1-3	AV	+
5	CHO TNFy 2.7	112995-P06	20nM	01.30.96	E1-3	AV	+
6	CHO TNFy 1.6	112995-P07	20nM	02.01.96	F1-3	AV	+
7	CHO TNFy 6.4	112995-P04	20nM	02.01.96	G1-3	AV	+
8	CHO TNFy 6.7	112995-P04	20nM	02.01.96	H1-3	AV	+
9	CHO TNFy 6.8	112995-P04	20nM	02.01.96	A4-6	AV	+
10		112995-P06	20nM	02.02.96	B4-6	AV	+
11		112995-P06	20nM	02.02.96	C4-6	AV	+
12		112995-P05	20nM	02.02.96	D4-6		
13		112995-P04	20nM	02.02.96	E4-6	:	
14		112995-P04	20nM	02.02.96	F4-6		
15	CHO TNFy 6.10	112995-P04	20nM	02.02.96	G4-6		
16	CHO TNFy 1.10	112995-P05	20nM	02.05.96	H4-6		
17		112995-P04	20nM	02.05.96	A8-10	· ·	
18	CHO TNFy 5.9	112995-P04	20nM	02.05.96	B8-10		
19				_=::=::			
20			<u> </u>				
22							
							. 1
23							
23					-		

- 7	
- · - ·	Timmunapræcipitation with TNF8- Monse-Serum
 .	January Januar
	Western with TNF Y-PPT - Mange Semin : Ver Ellie Bardaron
	1
	TITS TITY TIES ONLY
	ABAB AB AB
	A= Noos
	B=No,000
E 1	
\$ 2.5	
	Western (Ec. Netectia) TNE & PPT MOUSE Serm as THEY
Washington and the second	250m lac
1 6	2996
! !	
	Samples Pat were Tummes Precipilated:
	M.) CHOSSEMU - 1% aFBS
	2.) St 10.1 / Oi1696, - PO3
	3.) CHOTUES 1.5
	4.) CHOTUF 3 1.3
	8.) CHOTUE 391.7
1 1	6.) CHOTUE > 1.9
	2) CHOTUEY 27
	8) CHOTUES (16
	a) CHOTUES 64
- 	16) CHOTNES 6.7

	63
	100
(i) CWTUF8 6.8	
15) CHO LINER 5'8	
1 (b) C(0 (w) 2.1	
1.) dellute mouse antisonan (:10 (2 ne in 18 pe PBS)	(TIZE)
2) add in of 184 U. W. A. W. Jan Ja San & CHO - 114	
2) add 1 pl of the Uto de dillution to 500 pl CHO cold	ξ.
3.) marbale 30°, RT, on notator	
4.) Lass Prober G (Gamma burd Plus	
Sepanse Pamacia Coce Up. 17-0886-	-011
<u>∰ 1 </u>	
- Spin Inne of Protein of 5 see at 10000	Ipan I
aspirale supposed fill PBS	
- repeat 1x	
- resusper of Protein Bin PBS +0:17 BSA	<u>-</u>
5) add 20 jel Prolein R & Sample	
6) mensale for 30° PV potentor	
2) Spun 10-20 sac at 05 RPM	
asperate supervalents	:
8) cox 3 x 12 A 14 A DRO	
Sher compact Constant	
go to promote supported	•
9) edd 20 pl 2+ Sample buffer oul 10	: :
Spen after Soiling	
V V	
(0.) Transfer Supernola to who man Come do A Vica	
and frere at -20°C	
(> lord superson gel)	

es.

δij.

	65
	Egloblisting state 596 TUF & clones:
	expending of bedrap clones. (for freezing)
	Gwell: seep. 53
	- replace the tealum with MEMI, 97, diss, C. 18, 20 MARIN
20 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	Establishing of Slable 346 TNFY clones.
distriction of the state of the	Split confluent 125 blasses: 50 Y. gold in 775 flest in UEAL, 57 olf B. (7.65, 20~4M)X
	for freezing
1	25% Seed in T25 flage in MEHL, 5% OLFB, 1%B, 20 MMRK for yoshing, repose hearing crits CHO-8-SFM-CC+1%Sem
	1. 25% good in 6 wells of a Gwell distribus applification
	0,50,000,150,250,500 mM MTK (> 1, M - MTK stood solution!)
	7) 346 They 23 (112995 - pos)
	9) 346 TUFF 44 6 1129 98 - PO3
	5) 346 TUFY 4.6 (a29 45 -pas)) 1 1 1 1 1 1 1 1 1
121	9) 366 TUFS 5.3 (1129 95 - pos
The first of the second of the	

	<u>-</u>	<u>. </u>
66		; ;
Establishing of old CHO-TUFY clones.	:	
2.9.96 Proteingel and Electrohansfer for Class	lem 188648 .	<u> </u>
	V .	
(continued from p. 65)		
the supermodents were concentrated by	umenoprer poleti	u
and prepared like diented on p. 60 - 60	3	
- ran 2 groterigele 4-20 % NOVEX		,
- ran 2 groteingele 4-20 %. NOVEX		· :
53 37 70		<u> </u>
	· · · · · · · · · · · · · · · · · · ·	
		
Gel 1: 1) (1 (MOVEX prest. LWM)		
2) (3 CBOTUES	• • •	i I
3.) 1.3 -11-		
4.) 1.7 -11-		:
5): [9, -1-	i i	; ;
(6.) 2.7 -u-		
8-) mag control (ST(0.1)		! ;
9.) por contal 50mg The >		
(a) M		
		1 1
		++
Gel 2 1) W		++
2) 64 CHO TURS		1
3) 6.7		
4) 6'9 -u-1		
[10] Ineg contal! (CHO-9-SEND		
		++-
		- ! '

	67
	Western blot : Nover Victocallulage Membrane 12.12.96
	Vesten blot: Novek Vichocallaloge Hembrane 2.12.96
	- precent in 60 thing buffer !
	- elechobarster at 301, 16
	- The beooking 44. montest mulb
A 3 4 1	- 2 x 10'cres & ViA (x 983
	- 25h incubation with 1st AB. (Marge-shir TNFY-PPT
	ABOUL 1:5000 = 17.75 300 p. 62)
	- muse 34 with 1x PBS, west 3x 00 with (x PBS)
	- Ih in outsetion vig 2 and AB 1: 2000 (which was AB ARR)
	- nuise 3 x vit (x PBS ; weste 2 a 10' wiff (x PBS)
	- l'delection ECL
	- (see. exposure
	13-13-13-13-13-13-13-13-13-13-13-13-13-1
\$2.500 \$3.000	
	250
11: 12:	
· · · · · · · · · · · · · · · · · · ·	55 2 2
遊集	32.0
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/ toll wider	
5 SEE SEE	

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	250	
<u> </u>	a ce	
	20 Cd	:
	36	· · ·
<u> </u>	₩ 30-	 _
-: ·	6	-
		· -
	The second second	
2 12 07		·
2.12.96	CStablishing of Stable 346 Thit & Clanes	
	Crange Medium do CHO-S-SFM (+ (1. PS of B)	
<u> </u>	colly in 725 leasts for feeting (see p. 65)	:
	- remove be CM	
	0 1 15 49 (0)00	<u>; </u>
	- gold Sml of CAO-S-SPA VILLER Vo and	1 :
	725 losk	: :
	1:) 346TUF > 2.3/12995 paz	
	2) 34671471 411/11/29190	<u>; ;</u>
	5): 57.6TDF 7: 4.3 1.429.8 pez	
	5.) S46TUGS 1.8 (42995 po3	
	6-) 346 TUES 4.6 (429 95 002	
	6-) 346 TUE> 4.6 (1129 95 po3:	
	6-) 346 TUE > 4.6 (429 95 po 3 3) 346 TUE > 4.7 (429 95 po 3 4) 346 TUE > 4.10 (429 95 po 3 9.) 346 TUE > 4.10 (429 95 po 3	
	6-) 346 TUE > 4.6 (1129 95 po 3 7.) 396 TUE > 4.7 (1129 95 po 3 8.) 346 TUE > 4.10 / 1129 96 po 3 9.) 346 TUE > 5.3 / 1129 976 po 7	
	6-) 346 TUE > 4.6 / 429 95 po3 3.) 346 TUE > 4.7 (429 95 po3 4.) 346 TUE > 4.10 / 429 95 po3 9.) 346 TUE > 4.10 / 429 95 po3	
	6-) 346 TURY 4.6 (429 95 po3 3.) 346 TURY 4.7 (429 95 po3 4.) 346 TURY 4.7 (429 95 po3 9.) 346 TURY 5.3 (429 95 po3 9.) 346 TURY 5.5 (429 95 po3	

·	· · · · · · · · · · · · · · · · · · ·
	69
	CHO duko- D644 (010996) 03-14 2.1296
	CHO 2 14 DE 66 1 010 dd C D 03-16 1 5 15 de
	- passage colls. Speitfatio 1:10 and propose colle f. hasbetia
	- DATTER CONT. (C. AND DIVING CONT. INC. 12616)
	negropena 3ml of the collersp in 115ml
	Many L+, 5> OCES (1/1PS: 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
	sed int of this coll suspension in to exceed of
	(2 wells of a 2 6-well stills (1: 45 dil)
3	
	-> cells far hanslection:
[]	
	Compagation Cl. d. 246 875 - CNO 8710 1
	Compandice Stay 346515 - CHOSTIO.1 2.12.96
	- · · · · · · · · · · · · · · · · · · ·
	take 3x (me aliquois from each clone and passage in
	MEM L-, 5 x a ERS, 1 y PS, 80 M MIX
	1.) 396 815 1011696 pos (sparatio 1:4)
	2) CHO 3710.1/01/696 po8 (Splitmette 1:2)
2	Exablifung of stable 346 TUFY clones and (40 TUF) 2.13.96
1	
	treering of in process colle after about selection expanding !!!!
	of clones into 775 losses (see p. 65.)
	(L.) 346 9678 2.3/112995 po3
100	5) 346 LNEZ 4-1 /, 15662 bas
	3) 396 TNFY 9.3/42995 poz
A Company of the Comp	4.) 396TNF8 44/U2995 p03
	5) 346 TNFY 4.5/11/2995 poz
	(a) 3 46 7 NF 2 4.6 / U 2995 pg: 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
	0 26 (* 50) = 2 (11120 0) = 05
	10.) 3467NF3 54 / U2995 pag
	U.) CHOTUES 4.3 / 112995 pay
	12.) CHOTHES 4.4/429.85 pay
	

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314	71
	Long 24 well distes:
	6.) 346TUE> 6.1 (1002 CM
	7) 346.TUFY 6:2 (per 1)
	8) 346 TOFF 6.3 1 pag
	9.) 346 TNEY 6.4 (pag CM
	(0.) 396 TUF > 65/poz xx
	U) 3467NF> 6.6/p02
3 1 - 1	(2) 3467NFY 6.7 / par
	(3.) 346TNFY 6.9 / pas
	(5) 346TNFY 6.9 (pol
	(5) 346TWEY 6.60/par
-	Establishing of stable Chio MAS clones: 1 2.15.96
34241	
	Auphiliation; continued from p 58
\$ \$\$121-1 !	
	calls going at 0-1000mM MTR pooled and
	Seeded at 0, 100, 200, 500, 800 and 1000 mill WIX
	7) CAO LAD 2 1/5 (115654) 51/5 (115654) 51/5 (115654) 51/5
	5) CHOLME & (13 (1586) 608
5	13.) CHO THE DIG (12995 po8:
	(1) CHO TOPE 28(9 (U.29.9) pos 1
1 2 2	15.) CHOTHEY 2.7 / UZ995 608
	! wells gowing alt 10-400 mly pooled and see and at
	1 9, 50, 400, 7, 150, 250, 500 mm mix
	(x): CHO! INFX (1.6) (1.5664 b.0)
	(5) CHOILOTE 2: 5.3, (15dd 2005)
	3.) Cagrone 8 2 9 / 42995 poo
	G) CHOTUES 68/115440 DOF
	5) CHOTUET 6.4 (M2905 pa 6) 2 exchange TCM in
	6) CHATNET 6.2 (112995 06) ald chees same
	2 mon like older
	elanes (1-a)
	

		75	3 2.2
			[
4.47	Comparative study 396 STS. CHOST 10.	رح: رو	Q C
			1 1
			
	take 3x lml gliquots of and alone and persong cally:		Ti
	in MEML, 5% OFES, (2. PS, 80, MATER		
		1	11
The limit of the l			: .
展出	1) 346 STS / OIG96 POR	:	
	2) Cio St D (/ O ((96 P O 9		
1		<u> </u>	
		1	0-
	Comment of the state of the sta	2.20	-
	Comparation study 346875, CHOST 10.1	<u> </u>	·
	John Diller a constant all and all and all and	•	
	Jake 3 x 1 ml aliquots of each clone and passige color		
	in MEUL, 5% of EBS, LY-PS, 80,44 MIX	!	
	1.) 346 STS / OLIGAG PIO	<u> </u>	
	2) BRO STAP. 1 / OUGAG PLO	<u> </u>	
	2) Olo 21/0:15 out 6 DO		· : :
	Establishing of stable 346 VIGES clone	· ·	
4 35 4.			
	- amplification up to 8 xll MX		
	- passage colog and seed in 2 775 lastes		
	for freezing and analysis of color	:	
	in MEM 5- 20885, 18 PS, 80 MM MAX	. 1	· :
		: 1	:
	Establishing of stable 346 TNF & clones ! (continued)	p. ve	>)
		1	-::
	- expanding of backypelongs for freezing!!!		-
	prosege calls ham 725 lasks into AT 25 lasks		
	in MEMJ-, 5% alpes, 12. PS, 20mm into	\ : \ \	
- 1 2		!!!	!
	1) 346 TUE 8 4.2 / 112995 pot 7.) 346 TUE 86.6 / 112995 pog		!!]
	4.) 346 the 3 4.8/1129 35 po4 8.) 396 the 36.7/112995 po3		
	B.)3467WED 4.9/112995 poq Q.) 3467WEY6.9/112995 poz		
	(1) 346 LOES 21/115622 bad 1 10) 346 LAES 663 11565 603		
	13.13.10 1.18. 2.5 (11.5.6.2. bah 11.) 3.48 Line 2. 8. 10 \ 11.5.5 6. 10 \ 11.5.5 6.		
	6) 3481 M568 63/ 115682 bo3.		
		1-1-1	-+
			1

G	74		
<u></u>	1=		
i	7 000		· · · · · · · · ·
	2.20.96	- for two of the 346 The y comes the medium reas	:
É		replaced by MEMA, 5's. alogs, 14 PS, 80~M MIX	
E			; ;
L			
		2.) 346 TUF & 6.4/ 11 2995 p 02	:
<u> </u>			<u>:</u> .
			
	1 : 1	Tassaging of CHOdito DG44 cells	
	: :		-: :
·		1) passage collegen T 225 pages CHOOLING 1544/010496	: ic
	<u> </u>	Spect Made 1:X	.bre
ff		in MEMIT, 3% albs, 17.13 (2.20.96)	
ij	<u> </u>		
#		2) resuspend remaining colleger a final colone	
	: :	of Box (Ome	! :
	· · · · · ·	2000 m of Q 0 1 10 000	1 :
	1 1 1	resusperse 3 ml of this collegespersion in	
		3.7 the first (colline	<u> </u>
	1	delat I me of this deletter to each of the	1 1
		gells at 2-6 well disting for transportion	<u> </u>
	: : :	San thursday	-! !
			
			
	1 1	Establishing of 812 the CHO TNEY cell lings	·
	+ + + 1		
		discourse in the second	!
		implification of clones: continued p. 7	
		alls growing at 13 1000 and 1108	1
			!
		The state of the s	1
		10, 100, 200, 500, 800 and 1000 my MAX	1 !
			<u>i i</u>
		2) CHOTHE 2 12 / 112995 DOQ	-
		12-) CHOTHE 8/11-3/ 11-3/ 11-3/ 11-3-	 ! .
	1 1 1	13.) CHO TURE 11.2 / UZ498; 509 1	
		4) CHO TUPS 1-91 491 6-11 8 141 OND (P)	1 1
	1 4 1	15) CHO TUF > 12.7 (129.95) 1009	1 1
	-;i		, ,
1:			:

cells growing at 0-500 mM MTK, pooled ag:
goeded at 0, 600, 200, 500, 800 and 1000 min Mix
1.6 / [12995p08.
5) CHOLNER #3 5.3 (1150 42 0 08 1
3.) CHOTHEY 12.9 ((12995 p. 08
T (40 TNF) 25 6-8 (UZ94500C) (1)
5.) CNO FN; FX 6.4 / ((2995 p07
6-) CHOTHEX 6-7 (112995 p.07
Establishing of new stable CHO-colling 2.21.96
1 1 Constant of Med State Cho Weeling 12.21.96
Translection of CHBdhlo-Day's cells with
expressionice lore
1.) CESP/HITTIG 78 1 Project Bac (16, 11600, (pc/ (Da. Supper))
2.) GDF / HITER 36 / Project Code HG 11000 / pc ((Ean Sapper))
3.) VEGF 2/ HOSBD 47/ Project Cock HG 00400/ pc (Jung-Stan-Ha) 4.) FGF-11/ HE9CC 44/ Project Cock HG 07000/ 001 (Jung-Stan-Ha)
4.) FGF-11/HE9CC 44/ Project Cole HG 0 7000/ DC 1 (3/14-Son HG) 5.) Control p. 1/18 only
6) contal no that solded to cells
The contract of the contract o
1- CHOOLIFE DG44/DG44/010996 p16 colls were sourced
for tanolections in so seeler at 9 6 well dis the day getie !!
calls were 20-80% conflicer &
- nemone TCM, was called (x conty PBS) nemous PBS
add and of Optimen in 13 to easy could
prepare for each transfection negotion in 18 celepata
760000000000000000000000000000000000000
A: GOIL GOLLEN
10 pl Lipskisky
2. Some aplinen, sng Expressionactor, a sng plils

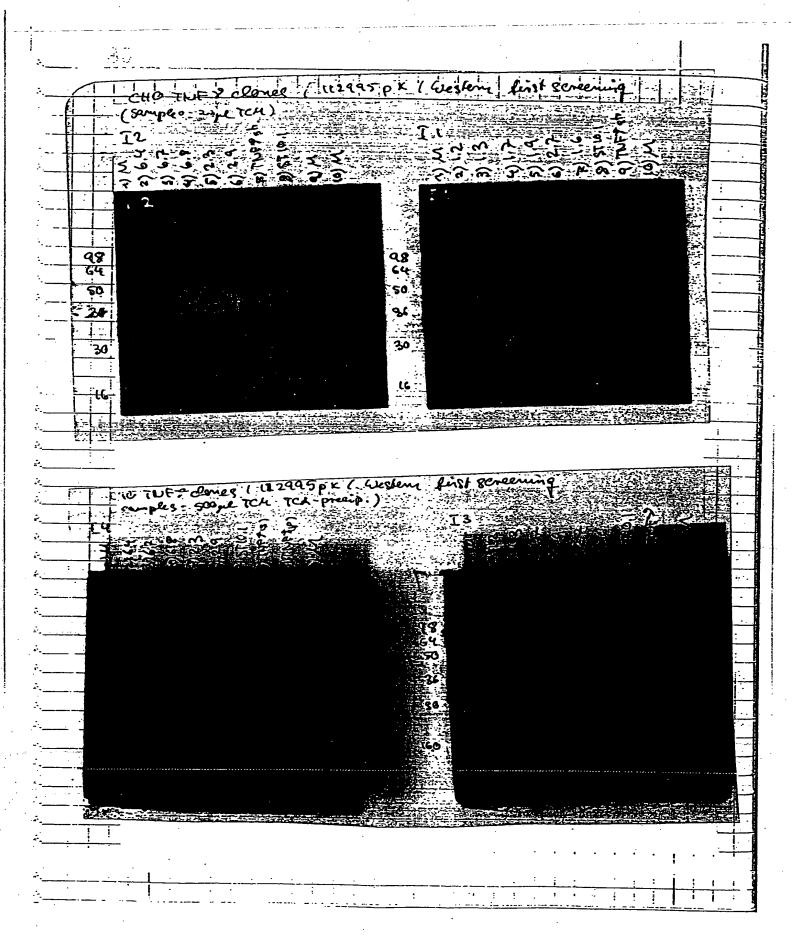
76	
1	· · · · · · · · · · · · · · · · · · ·
	- Mix A and B and mensele at Rt Ros 45
	- Add Transfaction wix for the adds and eigengale
	- dad (ml of OptiMEM + OF. alts + 18. PS
2.22.96	Establishing of CAO-THEY clones and 346-THEY clones
	Tritial screening for righ expressing cloner by
	Thitial screening for sight expressing cloner by litestern idralypes
: ; ;	- gample preparations: all TNF8 elones were invested
	Medium cox Spraester and frozen
i :	See p. 48 and p.56 for ChoTUFT clones se p. 70 for 3467488 clones
	-: 11 × CHO-TUF & clones Hat in parallel are amplified and one TUF & clones were rested for TUF & expression
	in Wastern Wagage
	Samples of each chare were loaded on a 16 %. NOVEKGEL
	a) 30 pl meaun sheight
	l) 500 jul medium TCA preapile Los

	- · · · · · · · · · · · · · · · · · · ·
	77
1	
	TCA - Preapplation:
1	- 500 pl supernale f + 50 pl 0.15 > 000
	+70 pl 00%. TCK
	- Correx 5 or see
	- remove sup gold coope checkens
	- 2'on ill
•	
	- resuspend pellet in 30 ne 0.11 wholf ho.17.505 - actal 10 me 4 x SDS lading buffer.
	- gold to be 4 x SDS togging buffer.
	positive contais: 1.) samples Baterer not precipilities (sheight)
	Dag E coli expressed a or reviel (M) (Mail Rob)
	(NES 495 2010kg to Dil at
1	CHO-2-SEN-II + One some prefer
	2) preipilabed somples: (precip.)
	50 ng TUFY 400 solded to 500 gl
	CHO-9-SEM-IT and the TCA pricipit.
	jour done as auscrise à associe
	I CHOTHEY clones - all gels IGY. WOVEX gels
	[CHOTHEY clones all gels IGY LOVEX gels
	el1:1)M
	2) CHOITHEY 1.2 30 pe shalpsi ?) CHOTHEY GU 30 pe slayst
	3) CHOTURY 1.3 -11- 3) CHOTURY 67 -11-
	4) CHOTHEY 1.7 -11- 4) CHOTHEY G8: -11-
	6) CHO FUEX 2.7 -11- 1 6) CHOTUEX 2.3 -10-
	F)CHOTUFY 1.6 -11- 3) CHOTUFY FT TO THE SCHAMES
+ 1	8)060 57 10.1 - u - 1 8) CHO STIOI 1 39 d 3 might
	9) 50 ng 7655 Staight 1 9) km
	(a) /\(\frac{\pi}{2}\)

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1 Clather Colones. 1 Clather Colones. 2 Chorer 12 Chorer 13 Chorer 14 Chorer 15 Chorer 16 Chorer 16 Chorer 16 Chorer 17 Chor	• •	· · ·		
(2) (A) 500 pleneplated 1) M 2) CHOTLET 12 (2) 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	78			
(2) (A) 500 pleneplated 1) M 2) CHOTLET 12 (2) 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2				
(2) (A) 500 pleneplated 1) M 2) CHOTLET 12 (2) 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2				
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2) CHOTLET 1.3 - 1 - 3) CHOTLET 6.4 500 per precipe (1) CHOTLET 1.3 - 1 - 4) CHOTLET 6.9 500 per precipe (2) CHOTLET 6.9 500 per precipe (3) 500 per p				
2) CHOTLET 1.3 - 1 - 3) CHOTLET 6.4 500 per precipe (1) CHOTLET 1.3 - 1 - 4) CHOTLET 6.9 500 per precipe (2) CHOTLET 6.9 500 per precipe (3) 500 per p		C203 1	- C- Q (1)	_]
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(1) CHOTHET! 3 - (1) (1) CHOTHET! 3 STATE THE SOLUTION STUDY (1) CHOTHET! 3 STATE THE SOLUTION STUDY (1) CHOTHET! 3 STATE THE SOLUTION STATE THE S			3) CHOTUPY 6.7 SCOUL mee's	
5) CHOTHETIA -11 5) CHOTHETIZ SOME proje. 5) CHOTHETIA -11 - 3) CHOTHETIZ A SOME proje. 2) CHOTHETIA -11 - 3) CHOTHETIZ A SOME proje. 8) CHOTHETIA -11 - 3) CHOTHETIZ A SOME proje. 8) CHOTHETIA -11 - 3) CHOTHETIZ A PROJECT. 9) SOME THEY 30 L STANGAT A SOME THEY precipilated. 10) M 11 346 THET SOME SOME SOLE. 11 3 346 THETY 2 3 30 M STANGAT 3) 346 THETY 4 6 30 M STANGATH. 12 346 THETY 4 1 30 M STANGAT 3) 346 THETY 4 10 30 M STANGATH. 13 346 THETY 4 2 30 M STANGATH. 13 346 THETY 4 3 30 M STANGATH. 13 346 THETY 4 5 30 M STANGATH. 14 30 M STANGATH. 15 30 M STANGATH. 16 34 M STANGATH. 16 34 M STANGATH. 17 CHO ST. 10 . 1 30 M STANGATH. 18 30 M STANGATH. 19 M STANGATH. 10 M STANGATH. 11 M STANGATH. 12 M STANGATH. 13 M STANGATH. 14 M STANGATH. 15 M STANGATH. 16 M STANGATH. 17 M STANGATH. 17 M STANGATH. 18 M STANGATH. 19 M STANGATH. 10 M STANGATH. 11 M STANGATH. 11 M STANGATH. 12 M STANGATH. 13 M STANGATH. 14 M STANGATH. 15 M STANGATH. 16 M STANGATH. 17 M STANGATH. 18 M STANGATH. 19 M STANGATH. 19 M STANGATH. 10 M STANGATH. 10 M STANGATH. 10 M STANGATH. 10 M STANGATH. 11 M STANGATH. 11 M STANGATH. 12 M STANGATH. 13 M STANGATH. 14 M STANGATH. 15 M STANGATH. 16 M STAN			4.) CHOTLET (8 500 LL BNC D	
2) CHOTHETIC -11- 3) CHOTHET GOLD price. 8) CHOTHETIC -11- 3) CHOTHET Striggt Precipe. 8) CHOTHETIC -11- 3) FONG THEY Striggt Precipe. 8) CHOTHETIC -11- 3) FONG THEY PRECIPILATED CO. (C.) M. (O.)	·		S.) CHOTELTS. 3 SOONE OMICO.	
2) CHOST 10: 1 (1) 50 ng The 3 30 L stright (2) 50 ng The 3 stright (3) 50 ng The 5 2 3 30 L stright (2) 3 stripes 4.6 38 master (4) 3 stripes 4.6 38 master (4) 3 stripes 4.6 38 master (4) 3 stripes 4.7 30 master (4) 3 stripes 4.7 5.7 30 master (4) 3 stripes 4.7 5.7 30 master (4) 3 stripes 4.7 5.7 30 master (4) 30 master (4) 3 stripes 4.7 5.7 30 master (4) 30 master (4) 3 stripes 4.7 5.7 30 master (4) 30 master (4) 3 stripes 4.7 5.7 30 master (4) 30 master (4			6) CHOTUFY29 500, 1 pricip.	
2.) 50mg TUEY 30pl stright 9.) 50mg TUEY precipitated (0.) M (0.) M (1.) 346 TUES samples: (2.) 346 TUES samples: (3.) 346 TUES 2.3 30pl stright 2.) 346 TUEY 4.6 38 pl stright (3.) 346 TUEY 4.1 30 pl stright 3.) 346 TUEY 4.6 38 pl stright (3.) 346 TUEY 4.1 30 pl stright 4.) 346 TUEY 4.10 90 pl stright (3.) 346 TUEY 4.3 30 pl stright 4.) 346 TUEY 4.10 90 pl stright (3.) 346 TUEY 4.5 30 pl stright 5.) 346 TUEY 5.3 30 pl stright (3.) 346 TUEY 4.5 30 pl stright 4.) 346 TUEY 5.3 30 pl stright (3.) 360 TUEY 4.5 30 pl stright 5.) 346 TUEY 5.3 30 pl stright (3.) 360 TUEY 4.5 30 pl stright 2.) 346 TUEY 5.3 30 pl stright (3.) 360 TUEY 4.5 30 pl stright 2.) 346 TUEY 5.0 mg stright (4.) 346 TUEY 4.1 30 pl stright 4.) 346 TUEY 5.3 30 pl stright (5.) 360 TUEY 4.1 30 pl stright 4.) 346 TUEY 5.3 30 pl stright (6.) M (6.) M (7.) M (3) CHOLDELI'P	+) CHO ST (O. (SCOPE precio	1
(0) M	•. •			_
3.) 346 TUES Samples. Gel 1: Gel 2: 1.) M 2.) 346 TUES 2 30 pe staget 2) 346 TUES 4.6 38 pel staget 4.3 346 TUES 4.6 38 pel staget 4.3 36 pel staget 5.) 346 TUES 5.7 36 pel staget 4.5 30 pel staget 5.) 346 TUES 5.7 36 pel staget 5.) 346 TUES 5.7 36 pel staget 5.) 36 pel staget 5.) 36 pel staget 6.) 36 pel staget 6.) 36 pel staget 6.) 36 pel staget 7.) 36 pel staget 6.) 36 pel staget 7.) 36 pel staget 7	• • • • • • • • • • • • • • • • • • •	(1) oned (no) some dividing		-1
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4.) 346 TUFF 4.3 39 LL GOLDS 4.1 346 TUFF 4.10 90 LL GROUNT 5.) 346 TUFF 5.3 30 LL GROUNT 5.) 346 TUFF 5.3 30 LL GROUNT 5.4 39 LL GROUNT 5.4 3		3.) 346 THEY 4.1 38 al shelpt		-
5.) 346 TUFF 4.5 39 L graph 6.) 346 TUFF 5.3 30 L ghalfet. 1.) 346 TUFF 4.5 39 L graph 6.) 346 TUFF 5.4 39 L ghalfet. 1.) CHO ST. (0.1) 30 pl graph 7.) EMELIFIED CHOSTO [30 pl gh.] 8.) Dang TUFF graph 8.) 21 L Graph 30 pl ghalfet. 1.) M. (0.) M. (1.) M. (2.) M. (3.) M. (4.) M. (4.) M. (4.) M. (4.) M. (5.) M. (6.) M. (6.) M. (7.) M. (7.) M. (7.) M. (7.) M. (8.) M. (8.) M. (8.) M. (8.) M. (9.) M. (9.		4.7346THEF 4.3 38ME GOSSI		-
6.) 3% TUFF 4.5 39 L Starkt 6.) 346TUFF 5: 4 30 M Sharkt 7.) CHO ST. (0.) 1 30 m L starkt 7.) CHO ST. (0.) 1 30 m L starkt 8.) 20 m g TUFF starkt 50 m g TUFF stark 24 Q.) M Q.) M Q.) M	: ; ; ;	5.) 3467WEF 44 :39 L down		-11
7) CHO ST. (0.1 30 pl shaifst ?) @ CHO ST (0.1 30 pl sh. 8.) Dang. TUES staifst 8.) @ CHO ST (0.1 30 pl sh. 9.) M 9.) M		16.7346 THET 4.5 SOME GRAPH	6) 346THF 5:4 39 MCHOWSH	
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i .	4.) 396 TUFY 4.3 900 precipit. 4.) 396 TUFY 4.10 900 ml precip
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<u> </u>	6.1 36 100 0 1.3 Descript 6.1 376 TUES 5.9 SOO L. pred A
•	7.) CHO ST 10.1 900 L precipit. 7.1 CHO ST 101 989 L precipit
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i i :	- Day cot 4 /a
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	- In blooking of 4°C (44. mile singercook mile
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	[173 (see p. (2) (:5000)
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	- mise 2 x with (MBS and cust 3x with 1x PBS, 10"
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	Result Wastern : W needs do Se aptimized Translettonting
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1 1 2	1) - background reduction use Theteli-PPS
11.	2.) - first AR incryation goes mig Gx
	B) - with sody specifity 1 &B was derive or four & call expressed
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82	
2.2396	Establishing of slable CHO clones:
	Courts way of stayee cas ownes;
	CESP, GDE, WEREZ FREII
	CESP, GDE, VEGEZ, FGE 11
	continued from P. 75
	Courted Paris (1. 7)
	Selection of reconforment CHO - clonge day dune 15-16
	Selection of reconficient CHO- clones for duplikation
	1-C000 120 x 100 100 100 100 100 100 100 100 100
	- Colls Datwere fanslected in Gwell Olises (500 p. 75)
	Can II will lange (c)
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	Flyhndema paling o
	1.) CESP-1 / 055126 DOI
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	3.) CESP-3 / OZZIRG pol
	5.7 CC31 3 7 GC21 to por
	4) GDF-1 /022196p01
	5) RDF-2 / 022196 p01
国	6) 805-3 (055166 ba)
	97 9700 3 7 0525148 1001
	7) VEGE 2-1/022196 001
	3) VEGFZ-1/022196p01
	A 2 2 3 3 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5
4	4) UEGF2-3/022196 POI
	10.) TGF-11-1 / B22196001
	15) 18 -11 -5 105516 bal 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1
	w-, who at 2 / Occito 1901
	B) negative compart 1/18-1
	14
N I I I	10.1
7.	(100) - 1-11 - Will - 3
	16) -11- (no Daile)
*	indicate the state of the state
` - 	1,4) 1 -12 (you gon) 1 1 1 1 1 1 1 1 1

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		83
Calabi	1.0	:
a.j .:	CHOdul- 70G44/	2.23.96
ا المناس	oth &	
771000	CHO other Dayy oroac ply spet 1.8	
	passage into MEML, SY-OEBS, 17. PS	
	(2.15) (2.15)	
		 .
	Ol Inchine T	1-21
	SUPERVISOR- Jun	the E-hang
	DATE- 2/23 fg	
	Comparation Strong 346 STS COOST 10.1	5.53.0E
<u>: </u>	•	·
: i i :	split 1:3 in Mend 5. diss, 12B 8022 12	2
	2) CHO St 10 1/01/696 pl (there are 229	.૧૯)
	8) GHO 21/01/01/06/0 Pil : -11-)
	Eslablishing of slash 346 VIGES alone	
-		
	- amplification up to 80, 4 MIX	
-	passage celler and good in 2 x 725 lasso	
	do dreesing and andyging	
	214266	
	Establishing of slable CHO clones 346THEZ	23996
	(continue & from p: 73)	
	beening of heads of	
	treezing of bedrup comes	
	1) 346 THEY 6.8 p. 03 6.) 346 THEY 4.2 / 112995 p. 04	
	2) 346 TUPY 6.9 p. 03	
	3-) 346 LME 26.10 603 : 8.) 346 LME 26.3 (115442 03)	
	4) 346 [NF749 pog : 9) 346 [NF 8:66 (11299 p) 33 5) 346 [NF 848 pog : 100) 1846 miss \$1/112998 = 1241	
	7 3 3 14 8 12 17 17 17 17 17 17 17 17 17 17 17 17 17	
	11.) 340 LNEX 25 (18566 8 bod)	
		+ + + + + + + + + + + + + + + + + + + +

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2000		
2.24.96	Eslesliting stare CHO THE & coll ling	
· 	id of part as a second as a second	_]
	displifeation continued from p74,75	_[
	collegere growing of 100 - 1000 m M MIX	
	poole conflict wells at soull and loomer and	
·	Sed in 0;0,5; 1: 15; 3 and 5 MM Mith	- Juna
		į
	L) CHOTHER 2, 2, 1, 112995 0.09	Ě
	人) CHO たます 2,3,(ill2995 pog	_
· .	3) CHOLMER 13 \ ((56.62 6(9)	
·	4.) CHOTHEF 6.4 (112995 P8	
	5.) CHOTUEN (.7/ 11.5662 688	
·		<u>'</u> i:
		1
22640	Comparable Study St5 St 10.1	_
	Contracting St. 12.	
	1952 Br (ml algrobs each clone and passage	-
	coles 1:4 in NEW / 5% OUTES, CY BS, 80 M MYK	-11
	1) 346 SIS /01696p 12	
	2) CHO 3110.1/01.696 PS	4
	7 20 310 7 910 68 62	
		\dashv
-	CHO dula - (DK 44	-11
	CHOdicho /DG 44/ 010996 P 15 CM 1 10	山
i : : :	(4000th 10944/010996 P 15 Spet 1:10	H
1 1 1		-#3
	MEA T. 22-06-08 (1 1 1 2)	
	Smu Mix Slood solution:	
	JUEN L 57.06-68! (1/ PS) 5 m M MX Slock solution! - dissolve 5 loong MX in 44 ml MEG & Medium	
	JMM MIX Slock solution; - dissolute of loong MIX in 4third MEM & Medium - killer steinlike Grongs a 0.2 pm filler	
	JUEN L 57.06-68! (1/ PS) 5 m M MX Slock solution! - dissolve 5 loong MX in 44 ml MEG & Medium	
	JMM MIX Slock solution; - dissolute of loong MIX in 4third MEM & Medium - killer steinlike Grongs a 0.2 pm filler	
	JMM MIX Slock solution; - dissolute of loong MIX in 4third MEM & Medium - killer steinlike Grongs a 0.2 pm filler	

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Estatisting of stable 3th TWEY clamps 2.22.96 Limplification continued from p 72: Limplification continued from p 72: - colls goes at low density main dead cells in regards. - nemous supernotant and cross east well with 2 mil 188. - stad 2 mil of WEML Strates wills. - stad 2 mil of WEML Strates wills. Estatilisting stable CHO TWEY clange. 2.29.96 Continued p 84 jumplification. colls were growing at concertations latered. On assimptific. 1.) poole cells at all concerts from I may final column. o) - Seed I mil in east well at a Govell clist. at 0: 0.5 1: 15: 3 and 5 mil Mix. l) - seed [mil into a 725 flass in MEML! and expand for lessing lass asspected.
Estatisting of stable Stil TNE'S clones Limplification continued from p 72: - colls gover at low density many dead collection regionate. - nemove superistant and cass east with 2 me 188 - stad 2 me of MEML, 5. affis IV B y beast gible yo reason colls of all different wells. Establishing stable CHO TNE'S clones. Establishing stable CHO TNE'S clones. Continued p 8th itemplification. colls were growing at concentrations at food on assignment. I) poole cells at all concents from I poole cells at
Estatisting of stable Stit TWE'S clones 22296 Limplification continued from p 72: - colls gove at low density many dead cells in suggested. - nemove supernotant and cress each vell with 2 mel 188 - stad 2 mel of UEML, 5. at 85 148 yo each yell you neaver colls of all different velles. Establishing stable CHO THE? clanes 229 a6 continued p 84 implification. cells were growing at concentations, describing in the power colls at all concents thouse in a session of a
chuplification continued from \$72: - calls goin et low density many dead cells in sugardo. - remove supernatur and crass east well with 2ml 1883. - stad 2ml of Well & 5. atts 1/2 Ps sto east gold. - stad 2ml of Well & 5. atts 1/2 Ps sto east gold. - stad 2ml of Well & 5. atts 1/2 Ps sto east gold. - stad 2ml of Well & 5. atts 1/2 Ps sto east gold. - stad 2ml of Well & concerns from a state of the s
chuplification continued from p 72 - calls goin at low density many dead cells in sugar, a - remove supernature and crass past well with 2ml HBS - stad 2ml of WEUL S. Atts 1/2 PS showed guell you reason colls at are different wells Establishing stable CHO THE P clanes Continued p 84 Amplification calls were growing at concentrations are fixed in I) poole cells at all concentrations in Time final coolumn o) - seed 1 ml in each well af a Genell clist. at 0. 0.5 1; 1.5; 3 and 5 july 1154
chaplification continued from p 72 - calls goin at low density many dead cells in sugar, a - remove supernature and crass past well with 2ml HBS - stad 2ml of WEUL STATES IN PS your gold you reason colls at all different wells Establishing stable CHO TN= 8 clarges Continued p 84 shaplification calls were growing at concentrations described on as withink 1) poole cells at all concentrations in 7 mel final column o) - seed I ml in east well at a Genell elso; at 0.0,5 1; 15; 3 and 5 july 1154
cells goes et los density many dead cellour sugando - nemove supernstart and coss cas vell with 2 me 188 - idal 2 me of MEM & 5. at 18 18 8 be east quell do neover collo et cello the 7 clanes Establishing stable CHO THE 7 clanes Continued p 84 implification cello vene growing at concentration details in 1.) poole cello at all concentration details in 1.) poole cello at all concentration details in 1.) poole cello at all concentration de descent descentration de action de la concentration de action de la concentration de la concentrati
cells gow et low density many dead cellour sugando. - nemove supernstart and coss coas well with 2 me 188 - idal 2 me of MEMY 5. at 18 19. 18 do and well do recover colls at all different wells Continued p 84 idenplification cells were growing at concentrations as fixed in a signification 1) poole cells at all concentrations with the final columns - med final columns o) - seed 1 me in easy well of a General cliss. at 0: 0.5 1.5: 3 and 5 me MEME
cells gow et low density many dead cellour sugando. - nemove supernstart and coss coas well with 2 me 188 - idal 2 me of MEMY 5. at 18 19. 18 do and well do recover colls at all different wells Continued p 84 idenplification cells were growing at concentrations as fixed in a signification 1) poole cells at all concentrations with the final columns - med final columns o) - seed 1 me in easy well of a General cliss. at 0: 0.5 1.5: 3 and 5 me MEME
- remove supernotant and coast each well with 2ml 188 - Ida 2ml of MEML, 5r. at 18 like you well yo reasce colly at all different wells Establishing stable CHO THE Y clanes continued p. 8'4 idenphilization cells were growing at concentrations as between O and Supplied I.) poole cells at all concentrations un 7 ml final column o) - seed 1 ml in each well at a Govell clist at 0: 0.5: 1: 15: 3 and 5 ml MTX
- remove supernotant and coast each well with 2ml 188 - Ida 2ml of MEML, 5r. at 18 like you well yo reasce colly at all different wells Establishing stable CHO THE Y clanes continued p. 8'4 idenphilization cells were growing at concentrations as between O and Supplied I.) poole cells at all concentrations un 7 ml final column o) - seed 1 ml in each well at a Govell clist at 0: 0.5: 1: 15: 3 and 5 ml MTX
- Idd 2ml of MEML 5 rates 14 Ps Jo 2002 well yo reasses colles of all different wells Establisting stable CHO THE P clanes Continued p 84 Idenphilication colle were growing at concertations deferein O and Symmetry I) poole cells of all concertations in 7 ml final column; o) - seed I ml in earl yell of a Garell clist; at 0: 0.5: 1: 1.5: 3 and 5 int Metal L) - seed I ml into a 1725 fant in MEML
- Idd 2ml of MEML 5 rates 14 Ps Jo 2002 well yo reasses colles of all different wells Establisting stable CHO THE P clanes Continued p 84 Idenphilication colle were growing at concertations deferein O and Symmetry I) poole cells of all concertations in 7 ml final column; o) - seed I ml in earl yell of a Garell clist; at 0: 0.5: 1: 1.5: 3 and 5 int Metal L) - seed I ml into a 1725 fant in MEML
- Idd 2ml of MEML 5 rates 14 Ps Jo 2002 well yo reasses colles of all different wells Establisting stable CHO THE P clanes Continued p 84 Idenphilication colle were growing at concertations deferein O and Symmetry I) poole cells of all concertations in 7 ml final column; o) - seed I ml in earl yell of a Garell clist; at 0: 0.5: 1: 1.5: 3 and 5 int Metal L) - seed I ml into a 1725 fant in MEML
Establishing stable CHOTNEY clones 229.96 Continued p. 84 Juplification cells were growing at concentration detacein and supplification 1.) poole cells at all concents thing in 7 ml final column. 2.0, 0,5, 1, 15, 3 and 5 ml Mix
Establishing stable CHOTNEY clones 229.96 Continued p. 84 Juplification cells were growing at concentration detacein and supplification 1.) poole cells at all concents thing in 7 ml final column. 2.0, 0,5, 1, 15, 3 and 5 ml Mix
Establishing stable CHO TNEY clanes 229.96 continued p. 84 iduplification cells were growing at concentrations detaceum OandSinMMXX 1) poole cells at all concentrations in final column 7 ml final column 0) - Seed I ml in earl yell afa Garell clist 4+ 0: 0.5: 1: 1.5: 3 and 5 in M MXX L) - seed I ml into a 1725 part in MENI
continued p 8'4 idinplification cells were growing at concentrations before in OardSupport 1.) poole cells at all concentrations in 7 ml final column 0) - Seed I ml in early sell afa Garell cliss at 0: 0,5: 1: 15: 3 and 5 july MTX 1) - Seed I ml into a 725 plans in MENA
continued p 8'4 idinplification cells were growing at concentrations before in OardSupport 1.) poole cells at all concentrations in 7 ml final column 0) - Seed I ml in early sell afa Garell cliss at 0: 0,5: 1: 15: 3 and 5 july MTX 1) - Seed I ml into a 725 plans in MENA
continued p 8'4 idinplifies from cells were growing at concentations between Oard Julitat 1.) poole cells at all concents from in 7 ml final column 0) - Seed I ml in early well afa Guell cliss 9t 0: 0,5: 1: 15: 3 and 5 july MTX 1) - Seed I ml into a 725 flas in MENA
celle vere growing at concentrations between Oa as withtee 1.) poole cells at all concentrations in 7 mel final column 0) - Socol I mel in agos well af a Genell clist 4 0: 0,5 1: 15: 3 and 5 my MEMLT
celle vere growing at concentrations between Oa as withtee 1.) poole cells at all concentrations in 7 mel final column 0) - Socol I mel in agos well af a Genell clist 4 0: 0,5 1: 15: 3 and 5 my MEMLT
celle vere growing at concentrations between Oa as withtee 1.) poole cells at all concentrations in 7 mel final column 0) - Socol I mel in agos well af a Genell clist 4 0: 0,5 1: 15: 3 and 5 my MEMLT
1.) poole cells at all concertations in 7 ml final column 0) - Seed I ml in early yell of a Garelle clist; 9 t 0: 0,5; 1; 15; 3 and 5 in Mits 1) - Seed (ml into a 725 flass in Mits)
1.) poole cells at all concertations in 7 ml final column 0) - Seed I ml in early yell of a Garelle clist; 9 t 0: 0,5; 1; 15; 3 and 5 in Mits 1) - Seed (ml into a 725 flass in Mits)
1.) poole cells at all concertations in 7 ml final column 0) - Seed I ml in early yell of a Garelle clist; 9 t 0: 0,5; 1; 15; 3 and 5 in Mits 1) - Seed (ml into a 725 flass in Mits)
7 ml final colume 0) - Soed I ml in early yell of a Gaiell cliss 9+0:0,5:1:15:3 and 5 my Mrx 1) - Soed (ml into a 725 flass in MEHX!
7 ml final colume 0) - Soed I ml in early yell of a Gaiell cliss 9+0:0,5:1:15:3 and 5 my Mrx 1) - Soed (ml into a 725 flass in MEHX!
0) - Seed I'ml in early well af a Garelle clists: 97 0: 0,5: 1: 15: 3 and 5 in MEHA!
1) - seed (ml m/o g 725 flas m MEH)
1) - seed (ml m/o g 725 flas m MEH)
l) - seed (mel into g 725 pars in MEHX!
1) - socol (mel into g 125 pars in HE4)
1) (NO THE + 23/ 112005-10. *)
2) CHO TUEY 23 (112995 DU)
3) CHO (NE) 1 31/ 11 2995 pa
4.) CHO THE 7: 6:4/ 12 29 95 09 1 1 1 1 1 1
5) CHO TUEY 6.7/ 11 2995 pag 111111111
2:2390 000

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	87
2 2 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	9) 3461 MEX 23/ 113682 600
	10.) 346 TWF8 5.4/ 42995 pos
	Comparative Steely STS ST 10.1
	Configuration Section 1
	Jake 3 x me glignots eger clone and passege celès. 1.3 in Mem d. 5% alti 12 P3, 80 x r mix
	1) 396 St5 101696 p B
	2.) 3HO STULLY OTTEGE & 13
	duplification of VIRES:
	180 M NT
	(der treezing a or la seling)
	Comparation Study at spring & VIGIF and TURY
	Company Study at social Ville and TUFY Clones (Loseling cuts 555-Cys , 35, Max)
	Than clones i freezing date:
	11.) STS (100, MM/X): 10.5.95 1355 (030196pol)
	31) VIGEZ (100, 12 100, 12. 3.85 VIGEZ (930186 pol)
	Sord colleg in 1725 at Round mage 11 Mill
	CHO die Daired Colodar bir Bort 1:101
	Men! L+, 5% after, 1% PS
	3,196 012
A CONTRACTOR OF THE PROPERTY O	

88:		
MGF3 received from idleric goodeal in		
75 for labeling:		
The state of the s	1	
1 11 VICEZ 10:15	1 ! !	
		1
Laseling of St. VIG E and The & co Coling		
3 4 96 Prepare cells for laseling expresiment:		
- Sells were seeded in 775 flasts before	:	
! (& previous pages)		1
to lateling experiment seed cells into the wells		
or a 6 well oust in Mond S' SI SIBS, () PS	· · -	
7>5-(276: delalo into 6 well		
1 361 870 1032186 63		1
1 1 2-) VIGES (5,M) /030146 p02 4008 1:10	1 !	#
3.) UIGES (100pH) 1 807. 1.8	· :	
1 S.) NIGES / 030(06 p 05 , 80% (1.8)	:	
6.) CHOTHER 5.7/115492 615 50% (15)		1
2.) Cionue's 4.3 / 1129.95 p 12 30% 1:3		
1 8 CHOTUFY 6.7 (1.5495 10 . 30). 1.5		
1 4.) CNO FOES 6.7- 11843: p-10		-
Composehice Stroly STS; ST 10.1:		
		
the 3x (me igliquets of east clone and passing		
celos 1:4 in MEHI - 5% OLERS (VPS 80 am letter		
346 8T 5 / BYIGAG DILY		#-
CHO STIGN (1011(96) PIG ! ! ! ! ! ! ! ! !	- - -	
	<u> </u>	-
	- -	-
	<u>:</u> :	

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1		96
		89
	Establiship glable 346 This & cell engs	1
	CSAIRESTANT JOINES 316 (DE 8 CORE STATES)	3.5.96
	continued from p. 86	
	cells growing from 0 - 1000 ml MTX cells	
	are confluent at soom u and intil	
	posts alle at 800mm + 1 m a or seed on 606	
	wells of 9 6 well at 0/0.5/ 1/1.5/3!	
1 1 :	and 5 pll.	
1 !		
	1.) 346 THES / 112995 POG / 2.3	
	2.) 346 TNE > 1 612995 POG / 4.1	
	3.) 346 TNEY / UZ995 POG / 4.3	<u>.</u>
	4) 346 They / 112995 606/ 4.9	
	6) 346 TNEX/ 112995 606/ 4.5	
	2) 346 TNEX/ (1298 126/ 4.7)	
	8) 346 TUES/ 112995 POG/ 4.10:	
	47 5	
- ■ • 1	(2) "546 TNFX/ ((2445 00c/: 5.3!	1 1 1 1 1
	10) 346 TNF>/ (12985 pag/ 5.4	
		3:5:26
	Exastisting stable CHO THE & all lines	3.5.26
	Establishing stable CHO THE & cell lines	3:5:26
	Exastisting stable CHO THE & all lines	3.5.26
	Establishing stable CHO This & all lines Continued from p. 86 Cells going in to to Inth Mex	3.5.26
	Extablishing stable CHO THE & all lines Continued from p. 86 Cells growing not to I p.M. M.K. poole cells at different concentrations and separ	3.5.26
	Establishing stable CHO This & all lines Continued from p. 86 Cells going in to to Inth Mex	3:5:26
	Extractional stable CHO INF & cell lines Continued from p. 86 Cells growing risk to I p.M. M. R. poole colls at different concertations and soul in 010.5/1/1.5/3 and 5 p.M. Mrx	3:5:26
	Extensioning stable CHO This & all lines Continued from p. 86 Colls going it to Inth Mex poole colls at different concentrations and soul in 0/0.5/1/1.5/3 and 5 p.M. Mex 1) CHOTUS 1.7/112995 p.10	3:5:96
	Continued from p. 86 Continued from p. 86 Colls gaving it to Inth Intal poole colls at different concentrations and second in 0/0.5/1/1.5/3 and 5, M Mrs. 1) CHOTUES 1.7/112995 p. 10 2.) CHOTUES 6.8/112995 p. 10	3.5.26
	Extractional stable CHO THE & all lines Continued from p. 86 Cells growing in to Inthe Mexican grows send in 0/0.5/1/1.5/3 and 5 in MAX 1) CHOTHER 1.7/112995 p.08	3:5:96
	Continued from p. 86 Continued from p. 86 Colls gaving it to Inth Intal poole colls at different concentrations and second in 0/0.5/1/1.5/3 and 5, M Mrs. 1) CHOTUES 1.7/112995 p. 10 2.) CHOTUES 6.8/112995 p. 10	3:5:26
	Continued from p. 86 Continued from p. 86 Colls gaving it to Inth Intal poole colls at different concentrations and second in 0/0.5/1/1.5/3 and 5, M Mrs. 1) CHOTUES 1.7/112995 p. 10 2.) CHOTUES 6.8/112995 p. 10	3:5:96
	Exestisting state CHO THE & all lines Continued from p. 86 Colls graving it to Int. Int. poole colls at different concentrations and scool in 0/05/1/15/3 and 5 mm Mr. 1.) CHO THE 1-7/112995 p.08 3.) CHO THE & C.8/112995 p.09 3.) CHO THE & C.8/112995 p.09	3.5.26
	Continued from p. 86 Continued from p. 86 Colls gaving it to Inth Intal poole colls at different concentrations and second in 0/0.5/1/1.5/3 and 5, M Mrs. 1) CHOTUES 1.7/112995 p. 10 2.) CHOTUES 6.8/112995 p. 10	3:5:96
	Exestisting state CHO THE & all lines Continued from p. 86 Colls graving it to Int. Int. poole colls at different concentrations and scool in 0/05/1/15/3 and 5 mm Mr. 1.) CHO THE 1-7/112995 p.08 3.) CHO THE & C.8/112995 p.09 3.) CHO THE & C.8/112995 p.09	3:5:26
	Exestisting state CHO THE & all lines Continued from p. 86 Colls graving it to Int. Int. poole colls at different concentrations and scool in 0/05/1/15/3 and 5 mm Mr. 1.) CHO THE 1-7/112995 p.08 3.) CHO THE & C.8/112995 p.09 3.) CHO THE & C.8/112995 p.09	3:5:26
	Exestisting state CHO THE & all lines Continued from p. 86 Colls graving it to Int. Int. poole colls at different concentrations and scool in 0/05/1/15/3 and 5 mm Mr. 1.) CHO THE 1-7/112995 p.08 3.) CHO THE & C.8/112995 p.09 3.) CHO THE & C.8/112995 p.09	3:5:96

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3.5.96	Laseling of St VIRF and Third celling
	ASSOCIATION OF STATE OF THE STA
	2) VIGES (5,M) 3) VIGES (10,M MTX)
	4) VIGES 1 630196 pos 5) VIGES 1 616
	(c) CHOTHEY 2.7 / 1/2995 p 12 3) CHOTHEY 4.3 / 1/2995 p 12
	9.) CHO THEY 6.7 (12995 pla
	alls are confuery in 6 vely of 34
	add to each world:
	add: 35S Getene and 35S-Mathonene.
	5 pl of and At to over well (10,0,0,0)
3.6 96	Establishing of recombinat CHOTHEY clonics
	continued from P. 85 - lupplification
	cells are gowing at 0-5 p.M. MIX
	Toole cells in and seed in 6 gold st
	7.) CHO The 3 2.3/ 112995 p. 11 (poole all yells)
	3.) CHO THEY 64/ 113998 PT -11-
	5) CHO THEY 6.7 111.2998 P 10 (poole: 3, 14 +5, 14)

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	The state of the s	the second section of the production
CONTRACT!		
		191
	desting:	1 : :
	10051079	
		_ : :
	for Joshing in the anhaus assay cells of the	
	following clones were seemed in to \$ 125 lasts	
	in used, one, 5, aso, 11 Ps.	_ <u> </u>
		<u> </u>
	1.) CHOTHEY 2.7/112995 p12 (0.5 m Mix	!
	(x1x1x) (x12 x12 x13 / (12995 11) 5.7 x4 11 0 HD (2:)	;
	(S) CAN HARO) DI 28PS/11/2/3 X447 CB)	•
	4) (40 7 4:636.2/112998 0 10	
		3(95
	Eglesticking of slesse CHO THEY clouds.	ا ٠٠٠٠
3 1-91	->	
1.11		
	continued from p 86:	
	Contoures of the 12 do	
1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	100 M MXX 0.6010	
- 開発 (数) ター	cells were gowing of 0-1000 nM MXX before-	
2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2 2	(Normania distribution formati)	
	00 10 /	
	wells frat were growing at 300 nm 500 nm and I pM	rene
	pooled and secolod in 0/500/1060/1000/300	0001
	5000 M MX	<u> </u>
	(1.) CHOLMES 1.9 4 (156.38 B 10 ; 11 ! ! !	
	12.) CHOTHES 1.9 / 112996124	
4 一会一	3.) CHOTHES 1-5/ 115998 P 4	
7 7 7		
2	herano colla for daring:	
	1-4	
\$ 55 gg	cells goving at 200 m. Mix greve special into	
	o 725 Jas in MEMI O MIX 5% OLFRY LY!PS I	
	1 4 7 65 years on mong 10 111 (1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	
	for Josking in the articulal asself	
	for tosting in the articulal asset	
	1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	
	1.) CHO THES 1-6/115436 PLO	
Thirting and the	5) CMO, LMES (-5 (115656, 611, 1)	
	3.) COTUES (-2 (U2986 PU	
ا المسلم		1

97
1 Establishor stable 346 TUES cole lines 1312.96
4
continued boy p. 89
1) Hancest supernatario for antividal 2584
(Down Rednance
2.) pools cells and seed at 0-5 pt MX
(2 mos lime blece concerts.)
- Arat
t.) Howest for testing: (see p. 89)
1) 346 TDFY 23/ p06 (colleg were growing of)
9.) 346 TNF8 4.1 (1 pog 0-1, 11 mta lielene 9.) 346 TNF8 4.3 / pog secola in sellevicio Maxi
4.) 346 TUFY 4.4 / DAC
5.)346 TUTY 4.5/ pag
6.) 346 TUFY 4.6 (page
7.) 346TNF8 4.7/ pac 8) 346TNF8 4.10 (pac
(9) 346 TUES 5.3 / DOC
(a) 3967NEX 5.4 / pag
colls each clone growing in 6 well sot different
MX concernor. 1(0 + 8 x/1)
a hist submitt by a too sel of say
a hist submit to a too had group.
(2)) see at collegen new Could sais that amplification -
1)346TUFF ? 3/112995POP
2) 346TUFY 41 / 112995 POD
4) 346704-744/ 112999 200
5) 346708 845 (11.2995 600

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